Oct.12,1955

Dear Joshua,

Last month, and the beginning of this one were spent on a course of statistics I have organised and thus I was unable to answer you earlier. Thank you very much for your letter. Let me deal first with the part of this it that deals with the paper on resistance.

Journal. I would certainly agree for Genetics. Bacteriologists who may be interested can receive it as a reprint. The paper is certainly no less genetical than Luria and Delbrück's, which did appear on Genetics. Title: yours much better. Addresses of the author s: if you like to give as a heading both the Istituto Sieroterapico Milanese and your Dept., it would have the advantage of paying for my reprints. I have no idea how much they would cost, but I guess it would already be in the range where one likes to have them paid by some Institution. We may indicate in the acknowledgments that the work was done, or partly done, while I was working in your Department on a Rockefeller fellowship. Order of authorship : it is very kind of you to give me priority. However, I still believe that the idea was more important than the experiment and the idea was yours. Introduction . Your text is O.K., I have only some objection to the use of the word vehement, just because it is somewhat funny in Italian (your second line of first page). I have no substitute to suggest, so, unless you have some bright idea, it is just as well to keep it. Would you not quote, at the same place, also the Symposium on Adaptation of the Society of General Microbiology? I am afraid I have no exact reference, but just the proofs of the book. Your page 3. I have no special reason for preferring the difference in growth rate, as you do, or the relative growth rate of the mutant, as I did, except that the latter method was used for calculating the values in table 4 , and that perhaps the relative growth rate may be less sensitive to changes in environmental conditions than the difference in absolute growth rates. As to formula (1) of my paper, page 4; I agree it is a poor looking one, but perhaps it shows the derivation. The introduction of R_t instead of e^t permits to use directly experimental data, such as r_o, r_t, and R_t for the calculation of the k values. I have used the approximations you suggest for the calculation of the k-values, but the approximation is not good enough for the last two cycles, when r_t and r_o are relatively high. Also, I forget: the experiments do not give easily a clue as to absolute growth rates, but only of relative ones. I should like to suggest that your middlepiece, page 3, be substituted as given in my appendix 1 to this letter. This also contains a proposed change that would end the introduction. I felt one should add probably as a footnote, which is there given about the effect of fluctuation of the initial number of resistants in the inoculum.

Nothing else about the introduction. Now, about your remarks to the main body of the paper : I entirely agree about deletion of the S^r suppressor and I also agree about your addition on stability of the streptomycin resistant. It is absolutely right that best and fertile, or rather, as you suggest, positive, should be defined exactly as you suggest. p.7 o.k. p.8 one might perhaps add: "In reconstruction experiments with mixtures of streptomycin sensitive and resistant mutants of E.coli, Ceppellini (1954) obtained a very closely similar value.", to be added at the end of the last paragraph, page 8.

Table 4, Cycle 3. I am sorry there was a mistake. m should be 2, instead of 0.5, and therefore E = 6.5 . The k value is correct as given.
O.k. about all the rest concerning the epxerimental part. Answering your further questions about it : Table 9 does refer to table 6, as you indicate, and not to table 7. In table 9 it is/culture with the 814 which should have a double arrow (or better, boldface italics, as you suggest). Stability is being tested. The mutant is still resistant at the moment; I shall send you a note about it as soon as an experiment similar to yours is completed.
Discussion. I have a few comments about some points that do not seem clear enough. I do not understand exactly what you mean by the two sentences : (middle of page 1, discussion): To give the obtained result, etc. and : Whether environmental differences among the culture tubes etc. Could you explain more at length? As to your question on data, I am giving what I have about it in appendix 2.
Discussion page 2, middle : Your sentence : "When either method of indirect selection should appear to fail, it should at least be shown that it is applicable to the recovery of/already adapted clones artificially added to the tested population." I would add at the end of it (if this is really what you meant): "if the negative result of indirect selection has to serve as valid proof against preadaptation".
All the rest is o.k. About acknowledgments to Rockefeller, perhaps you will find a better wording than I suggested above. I am certainly grateful to them for having taken me to Madison. Your acknowledgments to NCI, NIH etc. are of course ok.

As to the rest of your letter, and in particular your mention of the possibility that I may take a job in the States. Needless to say, I am appreciating greatly your interest on my behalf. If a full professorship were available here, I think I would prefer it to one elsewhere, essentially because it* gives a great liberty, even if the salary is meagre (on American standards, and also, to some extent, on Italian standards). No full prof'ship in Genetics has been available since 1947, and it is not yet clear when one will be available. Therefore my willingness to stay here decreases with time. I have not fixed a critical point for its decrease, or rather I am still above the critical point. However, I should like to be kept informed of good opportunities, if any turn up.

I had forgotten : fig. 2 (or rather, what was fig. 2, and is now fig. 1) is enclosed. I have more copies if useful. I think the labels with words stuck on it will not be noted in the cliché; this is the standard way in which German journals used to prepare their figures for publication.
I have nothing interesting in the lab, or at least nothing sufficiently clear or safe. And you?
All the best to you and Esther, from the both of us.

Yours Luca

* pleases Alba and

P.S. I am waiting for the book. Thank you very much for the offer of dealing with the final manuscript of the Genetics paper

inoculated with more than one resistant mutant. Since the expected enrichments are calculated on the assumption that only one resistant mutant is inoculated into the positive tube, a correction may be necessary, but the following argument shows that the correction is small (and therefore was neglected). The proportion of tubes out of n inoculated with a single sample containing m bacteria and distributed equally to them (so that each tube receives m/n bacteria on average), which (on averaging different experiments) receive 0, 1, 2... resistant mutants is given by the expansion of the binomial :

$$\left(\frac{1}{n} + \frac{n-1}{n} \right)^m$$

We select only the "positive" tubes, and therefore we must correct the average number of bacteria per tube, m/n, taking only the positive tubes into account. The proportion of "negative" (e.g. non-positive) tubes is, from the above formula : $\left(\frac{n-1}{n}\right)^m$ and that of positive tubes therefore is $1 - \left(\frac{n-1}{n}\right)^m$; the expected number of bacteria per ~~fertile~~ positive tube is then :

$$\frac{m}{n\left[1 - \left(\frac{n-1}{n}\right)^m\right]}$$

which exceeds ~~one~~ 1 by a small amount, e.g. for $m = 2$, $n = 10$, the most extreme instance here recorded, the correction factor is 1.055.

† : see reference [this bottom of page App. 1.

[I hope what given in the footnote is correct; I should have a chance of getting a more complete reasoning before roofstage, anyhow.] If worth it, the latter may be given as an appendix.

Appendix 2

Joshua, you ask about more data or comments on the "heritability of the variance". This is all I have about it .
The jackpot culture (No.41), containing 10 resistants/ml was expanded by seeding 1 ml of it in 100 ml. of broth. The fresh culture was kept in refrigerator for 10 days. Exactly the same was done with culture No. 42, the 0 mutants control.

From each 100 ml culture, 10 new cultures were prepared, each by adding 1 ml to Penassay tubes of 7 mls. The whole cultures were then plated on streptomycin agar, and the following counts were obtained:

From cult.41: 135, 142, 181, 123, 177, 111, 138, 149, 146, 144; average 144
variance 461
$\chi^2_{9} = 28.8$ (!)

From culture 42: 0, 0, 8, 2, 0, 0, 0, 1, 0, 1 average 1.2
variance 6.2

A second enlargement of jackpot and of culture 42 were made from the 100 ml cultures, about 29 days later. Cult.41 gave an average count of 22 resistants /ml, and culture 42 an average of 0.3 (averages based on 3 plates).

Do you think you can use this data? If so, I leave to you where to introduce this information.